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METHODS OF HEALING IN SOME ALGAL CELLS

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Introduction

At the present time very little is known regarding the method of healing in mature plant cells. The researches of Kite ('13), Chambers ('17), and Seifriz ('18) have been devoted principally to invertebrates and to ova. Their interest was directed to the study of the structure and character of the cell contents as revealed by dissection, rather than to the later reactions to the injury. The repair of the injury and subsequent growth of a few ova have been recorded, but these studies did not involve the rebuilding of plant cell walls. The difficulty of piercing plant cell walls seems to have prevented work along this line.

Kite, in his initial work, dissected Spirogyra and found that

> The cellulose wall is enormously cohesive. It is cut or punctured with extremely fine Jena glass needles with considerable difficulty. The outer surface is covered by an almost invisible soft gel. . . . The protoplasm of plant cells is much more dilute or less rigid than that of animals.

Chambers used the ova of Fucus, which did not involve the study of wall or plastids.

Seifriz, in his studies of plant structure, avoided forms with definite cell walls, with the exception of pollen tubes, the walls of which are extremely delicate. In speaking of oögonia of Fucus, Seifriz states:

> If these oögonia are teased out at a very early age they can be entered by a sharp needle. Very soon, however, the outer wall (exochiton) becomes too hard to be penetrated.

Topler ('03) in some experiments on injured or sectioned cells of *Bornetia secundiflora* found that the protoplasm remaining in a wounded cell would form a new wall. The amount of protoplasm lost in his experiments was sufficient to cause the remaining protoplasm to contract from the old wall against the adjacent uninjured cell of the filament, where it sometimes assumed an abnormal shape. A new wall was then developed by this protoplasm, forming a new cell.

The present article deals with some of the reactions of mature plant cells when punctured.

Material

Both fresh- and salt-water algae were used in the following described experiments. The fresh-water forms were collected near Oberlin, Ohio, and grown in culture in the laboratory. The marine forms were studied in the Biological Laboratory at South Harpswell, Maine. The writer wishes to express her appreciation to the directors, Dr. J. S. Kingsley and Dr. J. L. Conel, for the interest and kindness shown during the summers of 1919 and 1920.

Any form that will live under cultural conditions can be treated by the following methods. This study is confined to Nitella, Chara, and a few of the coarser filamentous algae.

Methods

The material was mounted in water on a slide and punctured free-hand under a low-power objective. In order to locate a given puncture readily in Nitella and Chara, the distance of the injury from a given node was recorded; in the filamentous forms every alternate cell was punctured. After puncturing, the material was grown in small culture dishes; and careful daily observations were recorded for a month. It was found that punctures made free-hand, with a steel needle ground to a very fine point, gave satisfactory results. This method did not require the time necessary for the more elaborate technique with the Barber pipette holder. In puncturing a large number of cells, punctures of all sizes and depths were obtained. The amount of internal disturbance was estimated by the amount of protoplasm lost and by the movement of the granules in the interior of the cell.

Observations

Vaucheria. The wall of Vaucheria consists of a thin, resistant membrane which is so very pliable that it is difficult to puncture it without injury to the remainder of the filament. If the filament is very turgid, a quick, sharp thrust of the needle will penetrate the wall successfully. The protoplasm is somewhat viscous and the flow is slight. The protruding protoplasm does not diffuse into the water, but forms a globular mass filled with plastids.

In the nine filaments studied the punctured portion was not separated later by a septum from the main thallus, but healed normally.

The delicate character of the plants and the fact that the readjustment of the plastids, as described by Klemm ('94), renders it very difficult to locate the injury after twenty-four or forty-eight hours were the determining reasons for not continuing the study of this form.

Cladophora. Both marine and fresh-water forms of Cladophora were studied. The cell just beneath a branch was selected for a base, and from this base every alternate cell was punctured both in the main axis and in the branch. In the two or three species experimented with, the puncture healed readily, but the later response to the internal disturbance was so unusual that I have reserved this and allied forms for later study.

Chara. In Chara the leaves and cortical cells from the growing point to the mature nodes were punctured. Unless the puncture was very small

(17–34 microns), the escaping protoplasm immediately diffused in the water, rendering it very difficult to estimate the extent of the protoplasmic loss. In a few cases, when the hole was very small (17–34 microns), a delicate film formed over the globule of protruding protoplasm. This globule continued to increase in size, apparently by the absorption of water.

The only movement of chloroplastids loosened by the puncturing occurred within a distance of 34 to 68 microns from the margin of the wound. Even when the hole was very small, rotation of the protoplasm immediately ceased and was not resumed. The original turgidity was also lost and was not regained. This was clearly shown by the invagination of the cross walls from the pressure of the adjacent cells.

Nitella. The internodes of Nitella proved to be unusually favorable material for the study of punctured cells. By puncturing at intervals of five minutes, it is possible to puncture an internode several times without disastrous results. Cells less than one centimeter in length I have punctured six or seven times. Each puncture was followed by a momentary pause in the protoplasmic movement. Even when the puncture was large and deep, with a corresponding loss of protoplasm, the regular rotation was resumed in less than two minutes. After the sixth and seventh punctures the pause was slightly longer than at first. Ultimately the successive drains on the protoplasm result in the cell losing its turgidity; and when that occurs the wall bends in front of the needle, rendering it difficult or impossible to puncture successfully. Although the cell may not be sufficiently turgid to puncture, this condition does not seem to cause any retardation in the healing of the previous wounds. The large size of the internodes and the regular arrangement of the plastids, as well as the moving protoplasm, aid in estimating the effect of the wounds.

Each puncture results in a rapid outflow of the cell contents. The protoplasm does not diffuse in the water, but forms globular or ovoid masses enclosed by a delicate film just outside the opening (Pl. III, fig. 1). The flow is slightly spasmodic—periods of rapid flow interrupted by brief pauses resulting in the formation of a number of separate globules. The plastids and other particles carried through the opening continue to move outward until they are lodged against the enclosing film. At first this film is extremely delicate and can be detected only by careful adjustment of the light or by staining. Gradually the entire mass of globules assumes a brownish tint which brings the films into sharp contrast with the surrounding water. After a few days this protruding mass disintegrates and disappears.

When the cell is punctured, the chloroplastids immediately surrounding the opening are torn from the plasma membrane (fig. 1), and either pass through the opening with the outflowing protoplasm, or are carried along in the rotating protoplasmic stream. The amount of internal disturbance caused by the wound is impossible to determine definitely, but some slight impression of it may be gained by observing the movement of granules.

If the puncture is small, the plastids, loosened from the wall, and the other particles moving in the rotating protoplasm do not show any acceleration until very near the opening. If the puncture is large (250–300 microns), the outward flow is spasmodic, a rapid flow following short pauses until the movement ceases. When the puncture is large enough for such movement, the dislodged chloroplastids and the other particles from every portion of the cell are seen to move toward the opening, indicating a very general internal disturbance.

An accumulation of particles in the hole promptly forms a protoplasmic plug closing the opening. In most cases the forming of this plug is greatly facilitated by an accumulation of the "spiny bodies" so peculiar to Nitella, described by Goeppert and Cohn ('49) and by Overton ('90). These bodies form the foundation of the plugs. If the same cell is punctured a number of times, the number of spherical bodies finally becomes exhausted, and the openings are closed by plastids and starch grains only. The other structures described by Klemm ('94), Küster ('99), and Noll ('99), although occasionally seen, do not appear to form any essential portion of the plug. When the "spiny bodies" are lacking, the plug is formed primarily of plastids and starch grains.

Many young growing internodes (5 mm. or less in length) were punctured. These internodes are less turgid than the older cells, and are filled with a denser mass of protoplasm. In every case healing occurred and growth was resumed so that measurable growth was recorded in twenty-four hours.

Sections made from material killed fifteen minutes after puncturing do not show any indication of the formation of a membrane to cut off the injured protoplasm. The globular bodies forming the plug are crowded at that time into a compact mass closing the opening. The inner surfaces of these bodies are still imbedded in the cytoplasm of the cell, and their spiny projections are unmodified. Before this material was killed rotation had been resumed, and the cytoplasm had been moving normally for 10 to 12 minutes. As the particles in the flowing protoplasm come against the plug they are slightly checked in their movement; and frequently this results in a somewhat denser, thicker mass of protoplasm just within and beyond the opening. The remainder of the cell does not show any modification in its protoplasmic structure.

In sections of material killed one hour after puncturing, it is possible to detect the new membrane forming (fig. 3). This membrane is clearly defined near the cell wall, a short distance back from the opening, but is rather indefinite and indistinct toward the interior of the cell. The membrane does not develop as a uniform, symmetrical invagination, but as a very irregular, convoluted film, which may pass between two adjacent pyrenoids with their associated starch grains, or over one spiny proteid body and outside the next. There is no structural difference visible between the pyrenoids and proteid bodies excluded and those retained by the new membrane. After twenty-four hours the membrane is completely formed over the base of the plug, and a thin film of wall is developed. This new wall can be traced at this time only for a short distance from the opening, where it gradually grows thinner and finally disappears. It thickens very slowly, for after three days it is only slightly more pronounced; though by this time the membrane or, rather, the new wall layers appear to extend farther outward from the wound over the cell. Ultimately the new wall encloses the entire cell, and the healing of the wound is completed as in Chaetomorpha.

Chaetomorpha melagonium f. *typica* (Web. & Mohr) Kützing. This marine Chaetomorpha was also a very satisfactory form for study. The material occurred in great abundance among the fronds of *Chondrus crispus*, and was easy to collect at low tide. The large size of the cells, the character of the cell wall, and the fact that it grows well in culture were factors determining the selection of this species.

The cell walls are tough, elastic, thick, and striated; and their inner surfaces are very finely corrugated. The corrugations usually run in a slightly spiral manner from one end of the cell to the other. They do not appear to be formed by overlapping lamellae, such as West and Hood ('11) found in Trentepohlia, but are rather slight folds of the inner membrane.

The turgid condition of the cells and the elasticity of the walls are essential factors in piercing these tough, pliable walls. When the needle is withdrawn, the contents of the cell slowly flow out and diffuse through the water. There is no surface film formed over the protruding protoplasm, as in the case of Nitella and many other algae; but the protoplasm, together with the chloroplastids, starch grains, and other particles, spreads through the water until it is no longer possible to trace them. Removing the needle causes a slight current in the water, which aids in scattering the particles and also determines the direction of their more rapid dispersal (fig. 2). Finally the starch grains and plastids accumulate at the opening until a plug is formed and all movement ceases. This plug seems to harden, and thus effectually closes the hole; and the cell may be repunctured in forty-five minutes or an hour, according to the size of the hole. The great elasticity of the wall is doubtless a material aid in checking the outflow. The extreme elasticity of these walls is strikingly illustrated if a cell is cut across one end, when the wall frequently contracts until the lumen of the cell is reduced to two thirds or even one half its original diameter.

At the end of two or three hours all the exuded contents have disappeared with the exception of a small, indefinite, discolored mass immediately over the opening; finally, after two or three days, this also disintegrates. The internal plug is still evident, but gradually grows less and less pronounced until it is difficult or impossible to locate the smaller punctures. The larger punctures (200–300 microns) are still visible at the end of a month, although less conspicuous than during the first week.

Cells plasmolyzed forty-five minutes or an hour after puncturing show that a new plasma membrane is growing inward, cutting off a portion of the injured protoplasm (fig. 4, *b*). At the end of an hour the plasma membrane has not completely separated the plug from the protoplasm of the cell, as a slender protoplasmic isthmus still connects the two (fig. 4). Previous to plasmolyzing it is impossible to distinguish any membrane or separation of the protoplasm near the puncture; but in plasmolysis the contracting membrane, drawing the plastids and pyrenoids away from the wall and plug, reveals the region of its formation and the extent of its growth. That a new membrane is formed is shown by the fact that the old membrane is still visible, extending across the space from the new membrane to the puncture (fig. 4, *a*). The space within the old membrane and between the plug and the plasmolyzed protoplasm is filled with a very finely granular substance, similar to cytoplasm in appearance, in which there are occasional coarser granules (fig. 4). At this time it is impossible to determine definitely whether the new membrane lines the entire cell or not, but this seems hardly probable in view of the later developments in the cell. Ultimately the membrane completes its growth, separating the plug from the protoplasm of the cell. As it is possible to repuncture the cell before the membrane has completed its growth, the hardening of the protoplasm to form the plug must effectively close the opening. This ingrowing membrane occasionally will form an extra loop, cutting out a deeply imbedded pyrenoid with its surrounding starch grains and cytoplasm. It is difficult to understand why this should occur, since this excluded pyrenoid does not appear different in any respect from those immediately adjacent but within the membrane (figs. 5, 6).

After the new membrane has formed, the typical lamellose wall begins to appear. But as this wall follows the much convoluted membrane where it is pressed against the plug of pyrenoids and plastids, it is very irregular in outline (figs. 5, 6). It is very difficult to trace the new wall layers back against the old wall. Apparently the first layers gradually grow thinner as they extend back from the injury until they disappear (fig. 6, *a*). The later layers appear to line the entire cell. If the first layers are formed only over the plug and not uniformly over the entire cell, it would seem probable that they are formed by the newer portions of the membrane which is an outgrowth of the original membrane still lining the remainder of the cell. As the new wall increases in thickness it separates the old injured portion of the membrane from the new.

DISCUSSION

The protoplasm in the different species of algae varies greatly in consistency. All degrees of viscosity can be found, from the extremely thin, watery fluid of Chara to the dense, viscous, slightly yellowish mass in the marine Cladophoras. In some species the protoplasm fails to diffuse in the water when the walls are punctured; in others the contents diffuse rapidly. In one case, that of Chara, this diffusion is so rapid and perfect that it is impossible to follow the movement under the microscope; the flow is not checked and the cells die. In all other cases in which the contents diffuse in the water, the flow is checked by the accumulation of particles and plastids in the opening, and healing proceeds normally.

When the protoplasm fails to diffuse in the water the film formed does not seem like a plasma membrane, but more nearly of the nature of a contact film. This is suggested by the fact that this film does not function in any active healing process, although the enclosing protoplasm is in close communication with the protoplasm of the cell. Again a new plasma membrane is formed slowly within the cell by growth from the old membrane. The present experiment has not revealed the exact origin of this membrane; but it seems probable that, in puncturing, a certain radius of the plasma membrane is injured, and that the new membrane begins to form at the outer margin of this region as an outgrowth of the original membrane.

In many cases a cell was torn or cut near one end. In the majority of such cases the major portion of the protoplasm immediately escaped through the open end, resulting in the final death of the cell. In one or two Chaetomorpha cells, a portion of the protoplasm which failed to escape formed an ovoid or globular mass next to the injured cell. This mass of protoplasm was covered by a delicate film, but a wall never developed, as has been described by Topler in his experiments with Bornetia. The mass disintegrated in two or three days.

It is a little difficult to determine Seifriz's exact meaning in his discussion of his only experiment with a cell wall, that of the pollen tube, for when discussing the experiment he says:

> The membrane formed in repairing a tear is of the same character as the original. This is not true in those instances where the wall is of cellulose, as in pollen tubes and plant embryos; for the enclosing surface layer of protoplasm is but a transformed portion of the living substance, the result of an immediate conversion of liquid plasma into a rigid gel of greater molar concentration (Pfeffer, 1891, p. 194).

In this statement he has failed to draw a clear distinction between the cell wall, the plasma membrane, and the film formed over the exuded protoplasm.

From the experiments on the living algal cells which have nonmiscible protoplasm, it would seem that the film formed has no functional value, but later an internal membrane develops which subsequently forms a new cell wall. Certainly my experiments on the algal cell do not substantiate Seifriz's statement that

> Miscibility precludes the presence of a membrane. Normal protoplasm is always capable of membrane formation. Therefore normal protoplasm can at no time be miscible.

The formation of an internal membrane in the algae develops more slowly than the similar structure mentioned by Chambers, of which he writes:

> If a tear of the surface layer or of any part of the cytoplasm be so injurious that disorganization sets in, a film may form around the disorganized area, separating it from the sound cytoplasm. The recovery of an injured cell is only brought about by the formation of a membrane-like film which prevents extension of the injury. A succession of films may form, as one after the other they succumb to the steady advance of the destroying process; and the film which finally holds out may enclose only a fraction of the original cell, but what it encloses will be normal protoplasm.

In the algal cell it is impossible to feel that the new membrane cuts out all the injured contents, since the disturbance is very evidently felt throughout the cell, as shown by the moving particles and also by the fact that the protoplasm immediately within the new membrane appears as dense and disorganized as the portion cut off as a plug. Obviously this membrane cannot serve in checking the loss of protoplasm from the cell, as that movement has ceased some time previous to its formation.

The presence of an enclosing wall is doubtless an important factor in developing various divergences from the responses of an ovum. One of the most essential of these is the readiness with which the loss of protoplasm is checked by plugging the gap in the wall. Then, also, the wall offers an effective support for the new membrane and new wall.

The influence controlling the development and direction of growth of this new membrane may be similar to the stimuli acting in the case of spore formation as described by Harper ('99, '00) for Pilobolus and Fuligo. He found that

> There is no differentiation of hyaline zones or other special regions prior to the formation of the cleavage furrows. Furthermore the nuclei show no special distribution about the cleavage planes. It is quite common to find a group of nuclei on one side of a cleavage furrow, while they are lacking over a considerable area on the opposite side. There is no indication whatever at this stage that the nuclei exert any direct influence on the orientation of the cleavage planes.
>
> If we examine the protoplasm immediately in front of these cleavage furrows also, we find it without differentiation of any sort which would indicate the direction which the furrow will take.

After discussing the influence that the nuclei may exert on the later stages of spore formation, he states:

> On the other hand it is quite possible to assume that cleavage throughout is controlled by the cytoplasm, at first with little reference to the distribution of the nuclei, but later with special reference to the formation of uninucleated cells.

In the separation of the plug, the cytoplasm appears to be the only controlling agent, for there is no visible movement of the nuclei toward the injured region. In the multinucleate forms discussed, a nucleus is found occasionally near the injury; but as nuclei are distributed throughout the cell, some must inevitably be carried out through the opening and lost, while others would be near the puncture when the movement ceases.

Even if several nuclei are lost, a sufficient number remains to maintain the vitality of the cell. In a Spirogyra cell which was punctured the single nucleus was carried through the hole and no recovery followed. Although the hole was small and the protoplasmic loss slight, the cell failed to recover its turgidity.

In Vaucheria the extremely thin membranous walls give so easily under the pressure of the needle that it is very difficult to puncture these walls unless the cell possesses a high degree of turgor. This is also true of Spirogyra, although the wall is much thicker. The tough walls of Spirogyra are the most difficult of any I have attempted. On the other hand, the walls of Chara, Nitella, and Chaetomorpha have a certain amount of rigidity in their structure which greatly aids in puncturing. There does not seem to be any relation between the type of wall and the viscosity of the enclosed protoplasm. Chaetomorpha, with its heavy, striate wall, has a thin, miscible protoplasm, while Cladophora, with an equally heavy wall, has very dense, nonmiscible contents.

It has not proved possible to estimate the total amount of protoplasm which may be lost by the injured cells. That this loss may be very large in some cases is evidenced by the fact that Nitella cells can be punctured six or seven times in an hour and still recover. There are no visible ill effects from the loss of protoplasm, and future growth appears normal in every respect. If the culture becomes infected with either bacteria or molds, the punctured cells do not seem any more susceptible than the uninjured. Yet, if the plants are placed in an unfavorable environment, the injured cells are always the first to succumb, indicating that there has been a lowering of their vitality.

I wish to thank Professor R. A. Harper and Professor F. O. Grover for their interest and encouragement during the progress of this study.

SUMMARY

1. All but one of the algae studied are able to heal a wound.

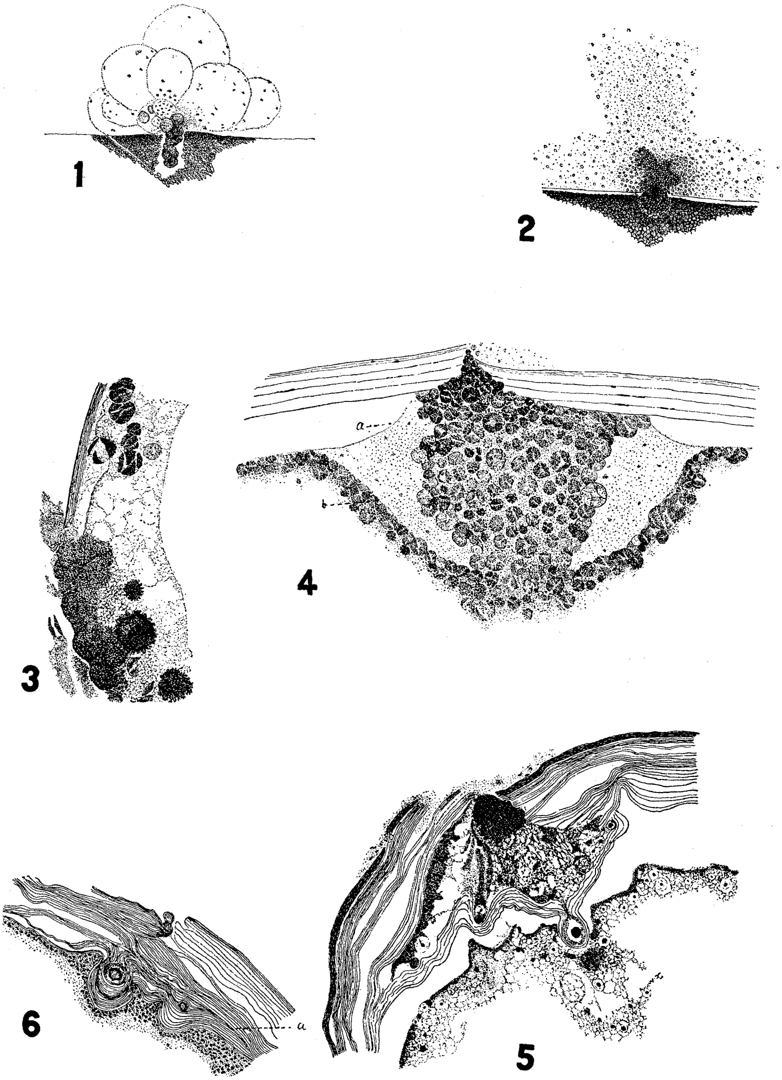
2. The density of the protoplasm varies from a very liquid condition in some species to a quite viscous condition in other species.

3. No correlation was discovered between the density of the protoplasm and the character of the cell wall.

4. The exuded protoplasm may or may not be miscible with water.

5. If the exuded protoplasm is nonmiscible, the film formed over the escaping protoplasm is not comparable with the plasma membrane.

6. The puncture is not closed by a film or membrane but by an accumulation of plastids, pyrenoids, and starch granules in the opening.



NICHOLS: HEALING IN ALGAL CELLS

7. A new plasma membrane grows inward from the old membrane and separates a portion of the protoplasm filling the puncture from the remainder of the cell.

8. A new wall is gradually formed by this membrane, and the healing is complete.

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EXPLANATION OF PLATE III

FIG. 1. Nitella with escaping nonmiscible protoplasm. × 160.

FIG. 2. Chaetomorpha with escaping miscible protoplasm. × 160.

FIG. 3. Nitella: section showing new membrane forming. × 160.

FIG. 4. Chaetomorpha plasmolyzed to show the new (*b*) and old (*a*) membranes. × 160.

FIG. 5. Chaetomorpha: section showing a new wall formed over the plug fifteen days after puncturing. × 160.

FIG. 6. Chaetomorpha: section showing a new wall well formed three weeks after puncturing. × 160.